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Validation of twelve chemical spot tests for the detection of drugs of abuse

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Abstract

Validation procedures are described for 12 chemical spot tests including cobalt thiocyanate, Dille–Koppanyi, Duquenois–Levine, Mandelin, Marquis, nitric acid, *para*-dimethylaminobenzaldehyde, ferric chloride, Froehde, Mecke, Zwikker and Simon's (nitroprusside). The validation procedures include specificity and limit of detection. Depending on the specificity of each color test, between 28 to 45 drugs or chemicals were tested in triplicate with each of the 12 chemical spot tests. For each chemical test, the final colors resulting from positive reactions with known amounts of analytes were compared to two reference color charts. For the identification of unknown drugs, reference colors from the Inter-Society Color Council and the National Bureau of Standards (ISCC-NBS) and Munsell charts are included along with a description of each final color. These chemical spot tests were found to be very sensitive with limits of detection typically 1 to 50 μg depending on the test and the analyte.

Keywords: Chemical spot test; Color test; Drug detection

1. Introduction

Chemical spot tests (sometimes referred to as color tests) provided toxicologists and criminalists with one of the earliest tools for the presumptive identification of drugs and

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poisons [1–3]. These tests continue to be popular for several reasons. They rely on simple chemical reactions and produce visible results that can be interpreted with the naked-eye. The reagents and laboratory materials needed to perform the tests are inexpensive and readily available. The tests can be performed by technicians without extensive training. Since the tests require minimal reagents and materials, small and even on-site laboratories can perform the tests. They can also be employed in the field by law enforcement agents. The utility of these tests is demonstrated by the fact that even today, when the use of sophisticated analytical instrumentation is so pervasive, they are still an integral part of the testing arsenal of forensic laboratories.

In two US National Institute of Justice (NIJ) standards, NILECJ-STD-0604.00 [4] and NILECJ-STD-0605.00 [5], the procedures for the use and validation of eleven different chemical spot-testing reagents were described. To better address the current needs of drug testing laboratories, the information in these documents was reviewed and updated or revalidated as needed. The need to include additional tests/analytes or remove existing tests/analytes from the original standards was assessed. An informal questionnaire addressing the use of these chemical spot tests in forensic laboratories was mailed to approximately 325 laboratories/individuals selected from the rosters of American Society of Crime Laboratory Directors (ASCLD), the Regional Association of Forensic Scientists and the Criminalistics Section of the American Academy of Forensic Sciences (AAFS). This mailing was performed in a semi-random fashion with an attempt to contact at least two laboratories or drug chemists in each state and to include the regional Drug Enforcement Administration laboratories. We received 121 responses. They indicated that chemical spot tests are still frequently used by 86% (104) of the responding laboratories. Greater than 90% of these laboratories used at least four of the tests; cobalt thiocyanate for cocaine, Duquenois–Levine for marijuana, Marquis for many basic drugs and *para*-dimethylaminobenzaldehyde (*p*-DMAB) for LSD. Ten of the chemical spot tests described in the NIJ documents were still routinely used by more than one third of the laboratories. Twenty-five percent of respondents suggested adding the Simon's or nitroprusside test for the detection of secondary amines, such as methamphetamine and methylenedioxymethamphetamine, to the battery of spot tests described in the original NIJ documents. Additional drugs that had become more prevalent since the publication of the standards such as acetaminophen, alprazolam, diazepam, ephedrine, hydrocodone and pseudoephedrine were added to the original list of analytes to be tested. This article reviews the data presented in the two original NIJ documents and presents new validation data for an expanded list of drugs using 12 chemical spot tests (CSTs).

2. Materials and methods

2.1. Chemicals

Cobalt thiocyanate, cobalt acetate dihydrate, glacial acetic acid, isopropylamine, acetaldehyde, ammonium vanadate, formaldehyde, *para*-dimethylaminobenzaldehyde, ferric chloride, vanillin, sodium molybdate, selenius acid, copper sulfate pentahydrate,

sodium nitroprusside, 2-chloroacetophenone and sodium carbonate were purchased from Sigma–Aldrich Chemical (St. Louis, MO, USA). Methanol, hexane and chloroform were obtained from Burdick and Jackson (Muskegon, MI, USA). Hydrochloric acid, sulfuric acid, nitric acid and pyridine were purchased from Mallinckrodt Baker, (Paris, KY, USA). Ethanol was obtained from Quantum Chemical (Tuscola, IL, USA). The drugs were purchased in powder form from Sigma–Aldrich Chemical (St. Louis, MO, USA), Alltech-Applied Science (State College, PA, USA) or Research Triangle Institute (RTI, NC, USA).

2.2. Materials

Porcelain plates with 12 wells, glass culture tubes (12×75 mm) and Pasteur pipettes were purchased from VWR Scientific Products (Denver, CO, USA). The Munsell Book of Color (Volumes 1 and 2) were purchased from GretagMacbeth (New Windsor, NY, USA). The centroid color charts, published by the Inter-Society Color Council and the National Bureau of Standards, were obtained from Nick Hale (Naples, FL, USA).

2.3. Chemical spot test procedures

The procedures for preparing the chemical spot test reagents and performing each test are described below. One or two drops of reagent(s) were added to the drug using a Pasteur pipette unless otherwise noted.

A.1 Cobalt thiocyanate

Dissolve 2.0 g of cobalt (II) thiocyanate in 100 ml of distilled water.

A.2 Dille–Koppanyi reagent, modified

Solution A: Dissolve 0.1 g of cobalt (II) acetate dihydrate in 100 ml of methanol. Add 0.2 ml of glacial acetic acid and mix.

Solution B: Add 5 ml of isopropylamine to 95 ml of methanol.

Procedure: Add two drops of solution A to the drug, followed by one drop of solution B.

A.3 Duquenois–Levine reagent, modified

Solution A: Add 2.5 ml of acetaldehyde and 2.0 g of vanillin to 100 ml of 95% ethanol.

Solution B: Concentrated hydrochloric acid.

Solution C: Chloroform.

Procedure: Add three drops of solution A to the drug and shake for 1 min. Then add three drops of solution B. Agitate gently, and determine the color produced. Add nine drops of solution C and note whether the color is extracted from the mixture to A and B.

A.4 Mandelin reagent

Dissolve 1.0 g of ammonium vanadate in 100 ml of concentrated sulfuric acid.

A.5 Marquis reagent

Carefully add 100 ml of concentrated sulfuric acid to 5 ml of 40% formaldehyde (formaldehyde:water, v:v).

A.6 Nitric acid

Concentrated nitric acid.

A.7 Para-dimethylaminobenzaldehyde (p-DMAB)

Add 2.0 g of *p*-DMAB to 50 ml of 95% ethanol and 50 ml of concentrated hydrochloric acid.

A.8 Ferric chloride

Dissolve 2.0 g of anhydrous ferric chloride or 3.3 g of ferric chloride hexa-hydrate in 100 ml of distilled water.

A.9 Froehde reagent

Dissolve 0.5 g of molybdic acid or sodium molybdate in 100 ml of hot concentrated sulfuric acid.

A.10 Mecke reagent

Dissolve 1.0 g of selenious acid in 100 ml of concentrated sulfuric acid.

A.11 Zwikker reagent

Solution A: Dissolve 0.5 g of copper (II) sulfate pentahydrate in 100 ml of distilled water.

Solution B: Add 5 ml of pyridine to 95 ml of chloroform.

A.12 Simon's reagent

Solution A: Dissolve 1 g of sodium nitroprusside in 50 ml of distilled water and add 2 ml of acetaldehyde to the solution with thorough mixing.

Solution B: 2% sodium carbonate in distilled water.

Procedure: Add one drop of solution A to the drug, followed by two drops of solution B.

2.4. Validation of chemical spot tests

2.4.1. Test color and specificity

The drugs and other analytes were classified and prepared as either a drug standard, crystal, powder, tablet, or extract (Table 1). Drug standards were prepared in either chloroform or methanol at a concentration of 2.0 or 4.0 mg/ml (free-base). Crystals were tested without further processing. Tablets were crushed into a fine powder and leaf

Table 1
The classification of chemical spot test analytes

Analyte	Classification	Analyte	Classification
Acetaminophen	Drug standard	MDA HCl	Drug standard
Alprazolam	Drug standard	Meperidine HCl	Drug standard
Amobarbital	Drug standard	Mescaline HCl	Drug standard
Aspirin	Tablet	Methadone HCl	Drug standard
Baking soda	Powder	Methaqualone	Drug standard
Benzphetamine HCl	Drug standard	Methyiphenidate HCl	Drug standard
Brompheniramine maleate	Drug standard	Morphine monohydrate	Drug standard
Chlordiazepoxide HCl	Drug standard	Nutmeg	Extract
Chlorpromazine HCl	Drug standard	Opium	Powder
Cocaine HCl	Drug standard	Oxycodone HCl	Drug standard
Codeine	Drug standard	Pentobarbital	Drug standard
Contac	Tablet	Phencyclidine HCl	Drug standard
D-Amphetamine	Drug standard	Phenobarbital	Drug standard
D-Methamphetamine	Drug standard	Procaine HCl	Drug standard
Diacetylmorphine HCl	Drug standard	Propoxyphene HCl	Drug standard
Diazepam	Drug standard	Pseudoephedrine HCl	Drug standard
Dimethoxymethaphetamine HCl	Drug standard	Quinine HCl	Drug standard
Doxepin HCl	Drug standard	Salt	Crystals
Dristan	Tablet	Secobarbital	Drug standard
Ephedrine HCl	Drug standard	Sugar	Crystals
Exedrine	Tablet	Tea	Extract
Hydrocodone tartrate	Drug standard	THC	Extract
LSD	Drug standard	Tobacco	Extract
Mace	Crystals		

material was extracted with hexane. For all of the CSTs (except A.3), 500 μg of each analyte (125 or 250 μl of drug standard) was added to each of three wells on the porcelain test plate. For the drug standards and extracts, the organic solvent was evaporated and the residue was reconstituted in 100 μl of chloroform before the CST was performed. The CST reagents were then added with a Pasteur pipette as described in Section 2.3 for each test. For A.3, 500 μg of analyte was added to a glass culture tube. If organic solvent was present, it was evaporated and the test reagents were added as described for A.3. The final color was observed after 5 min and compared to reference colors in the Munsell and centroid color charts. Each analyte was tested in triplicate.

2.4.2. Drug detection limit

A working 1.0 μg/μl solution (or lower if necessary) of each analyte to be tested was prepared. The limit of detection (LOD) for each analyte was determined by testing serial dilutions of the working solution until the lowest concentration of analyte that was detectable in five replicates ($n=5$) was identified. This concentration was then multiplied by ten and recorded as the 'operational drug detection limit'. All tests were performed in the porcelain test plates except for A.3 which was performed in glass culture tubes.

3. Results and discussion

Chemical spot tests are widely accepted as presumptive tests for drug detection. These CSTs provide information that allows the analyst to select the appropriate testing procedures to confirm the identity of the suspected drug. The information listed in the tables is intended as a guide for using CSTs and for preparing quality control materials for chemical spot tests when they are performed in the laboratory or in the field. The actual color produced by the reagents for each drug may vary depending on many factors: the concentration of the drug, whether the drug is in salt or free base form, which salt form is present, any additional diluents or contaminants present in the sample, the color discrimination of the analyst and the conditions under which the test is performed [2,6].

The original NIJ standards [4,5] used centroid color charts published by the Inter-Society Color Council and the National Bureau of Standards (ISCC-NBS) for color comparison. These charts include almost 270 colors logically grouped and listed numerically. However, these color standards are obsolete and are no longer considered to be an international standard for color description or comparison. Therefore, the ISCC-NBS numbers are listed for historical comparison purposes only. The ISCC-NBS charts have been replaced by the Munsell Color charts. The Munsell Book of Color (Volumes 1 and 2) is a master atlas of color that contains almost 1600 color comparison chips. The colors are prepared according to the specifications contained in the final report of the subcommittee of the Optical Society of America. Each page of the Munsell book presents one hue. There are 40 pages, each is 2.5 hue steps apart. On each page, the color chips are arranged by Munsell value and chroma. The standard way to describe a color using Munsell notations is to write the numeric designation for the Munsell hue (H) and the numeric designation for value (V) and chroma (C) in the form of H V/C. Since there are considerably more colors in the Munsell charts than in the centroid charts, two or more Munsell notations may correspond to the same previously used ISCC-NBS number.

Depending on the specificity of each color test, between 28 to 45 drugs or chemicals were tested in triplicate with each of the CSTs. For each CST, the final color resulting from a positive reaction with a known amount of analyte was compared to two reference color charts. These results are shown in Table 2. Reference colors from the ICSS-NBS and Munsell charts were included along with a description of each final color.

A positive CST may indicate a specific drug or class of drugs is present in the sample, but the tests are not always specific for a single drug or class. For this reason, laboratories must rely on a battery of CSTs for the preliminary identification of an unknown drug. For example, Cobalt thiocyanate (A.1) is used to detect cocaine. However, many other drugs will also react with this reagent (Tables 2 and 3) and each analyte that tested positive with cobalt thiocyanate produced a strong blue color. Also, the nitric acid test produced variations of yellow and orange colors with a variety of analytes including acetaminophen, diacetylmorphine, dimethoxymethamphetamine and mescaline.

Six of the CSTs are indicated for the detection of opioids and other amines. These include Mandelin (A.4), Marquis (A.5), nitric acid (A.6), ferric chloride (A.8), Froehde

Table 2
Final colors produced by reagents A.1 through A.12 with various drugs and other substances

	Analyte	Solvent	ICSS-NBS[g]	Color	Munsell
A.1	Benzphetamine HCl	$CHCl_3$	168	Brilliant greenish blue	5B 7/8
A.1	Brompheniramine Maleate	$CHCl_3$	168	Brilliant greenish blue	5B 6/10
A.1	Chlordiazepoxide HCl	$CHCl_3$	168	Brilliant greenish blue	2.5B 6/8
A.1	Chlorpromazine HCl	$CHCl_3$	168	Brilliant greenish blue	5B 6/10
A.1	Cocaine HCl	$CHCl_3$	169	Strong greenish blue	5B 5/10
A.1	Diacetylmorphine HCl	$CHCl_3$	169	Strong greenish blue	7.5B 6/10
A.1	Doxepin HCl	$CHCl_3$	168	Brilliant greenish blue	5B 6/10
A.1	Ephedrine HCl	$CHCl_3$	169	Strong greenish blue	5B 5/10
A.1	Hydrocodone tartrate	$CHCl_3$	168	Brilliant greenish blue	5B 6/8
A.1	Meperidine HCl	$CHCl_3$	169	Strong greenish blue	5B 5/10
A.1	Methadone HCl[a]	$CHCl_3$	168	Brilliant greenish blue	5B 6/10
A.1	Methylphenidate HCl	$CHCl_3$	168	Brilliant greenish blue	10BG 6/8
A.1	Phencyclidine HCl	$CHCl_3$	169	Strong greenish blue	5B 5/10
A.1	Procaine HCl[a]	$CHCl_3$	169	Strong greenish blue	5B 5/10
A.1	Propoxyphene HCl[a]	$CHCl_3$	169	Strong greenish blue	5B 5/10
A.1	Pseudoephedrine HCl	$CHCl_3$	169	Strong greenish blue	5B 5/10
A.1	Quinine HCl	$CHCl_3$	178	Strong blue	2.5PB 5/12
A.2	Amobarbital	$CHCl_3$	222	Light purple	5P 7/8
A.2	Pentobarbital[a]	$CHCl_3$	222	Light purple	5P 7/8
A.2	Phenobarbital[a]	$CHCl_3$	222	Light purple	5P 7/8
A.2	Secobarbital[a]	$CHCl_3$	222	Light purple	5P 7/8
A.3	Mace[f]	Crystals	237[b]	Strong reddish purple[b]	2.5RP 5/12[b]
			237[c]	Strong reddish purple[c]	2.5RP 5/12[c]
			221[d]	Very light purple[d]	5P 8/4[d]
A.3	Nutmeg	Extract	244[b]	Pale reddish purple[b]	10P 6/4[b]
			244[c]	Pale reddish purple[c]	10P 6/4[c]
			261[d]	Light gray purplish red[d]	5RP 7/4[d]
A.3	Tea	Extract	119[e]	Light yellow green	5GY 8/6
A.3	THC[a]	EtOR	204[b]	Gray purplish blue[b]	7.5PB 4/4[b]
			199[c]	Light purplish blue[c]	7.5PB 7/8[c]
			219[d]	Deep purple[d]	7.5P 4/12[d]
A.4	Acetaminophen	$CHCl_3$	107	Moderate olive	10Y 5/8
A.4	Aspirin	Powder	127	Grayish olive green	2.5GY 4/2
A.4	Benzphetamine HCl[a]	$CHCl_3$	116	Brilliant yellow green	2.5GY 8/10
A.4	Brompheniramine	$CHCl_3$	50	Strong orange	7.5YR 7/14
A.4	Maleate Chlorpromazine HCl	$CHCl_3$	108	Dark olive	10Y 3/4
A.4	Cocaine HCl[a]	$CHCl_3$	69	Deep orange yellow	10YR 7/14
A.4	Codeine[a]	$CHCl_3$	108	Dark olive	5Y 3/4
A.4	Contac	Powder	84	Strong yellow	2.5Y 6/10
A.4	D-Amphetamine HCl[a]	$CHCl_3$	164	Moderate bluish green	5BG 5/6
A.4	D-Methamphetamine HCl[a]	$CHCl_3$	137	Dark yellowish green	10GY 4/6
A.4	Diacetylmorphine HCl[a]	$CHCl_3$	43	Moderate reddish brown	10R 3/6
A.4	Dimethoxy-meth HCl	$CHCl_3$	96	Dark olive brown	5Y 2/2
A.4	Doxepin HCl	$CHCl_3$	44	Dark reddish brown	10R 2/4
A.4	Dristan	Powder	110	Grayish olive	7.5Y 4/4
A.4	Exedrine	Powder	108	Dark olive	7.5Y 3/4
A.4	Mace[f]	Crystals	125	Moderate olive green	5GY 4/8

Table 2. Continued

	Analyte	Solvent	ICSS-NBS[c]	Color	Munsell
A.4	MDA HCl	$CHCl_3$	193	bluish Black	10B 2/2
A.4	Mescaline HCl[a]	$CHCl_3$	78	Dark yellowish brown	10YR 3/4
A.4	Methadone HCl	$CHCl_3$	187	Dark grayish blue	5B 3/2
A.4	Methaqualone	$CHCl_3$	66	Very orange yellow	10YR 8/14
A.4	Methylphenidate HCl	$CHCl_3$	67	Brilliant orange yellow	2.5Y 8/10
A.4	Morphine monohydrate[a]	$CHCl_3$	47	Dark grayish reddish brown	10R 3/2
A.4	Opium[a]	$CHCl_3$	59	Dark brown	7.5YR 2/4
A.4	Oxycodone HCl	$CHCl_3$	103	Dark greenish yellow	10Y 6/6
A.4	Procaine HCl	$CHCl_3$	51	Deep orange	5YR 5/12
A.4	Propoxyphene HCl	$CHCl_3$	44	Dark reddish brown	10R 2/4
A.4	Quinine HCl	$CHCl_3$	100	Deep greenish yellow	10Y 9/6
A.4	Salt	Crystals	50	Strong orange	5YR 7/12
A.5	Aspirin	Powder	13	Deep red	5R 3/10
A.5	Benzphetamine HCl[a]	$CHCl_3$	41	Deep reddish brown	7.5R 2/6
A.5	Chlorpromazine HCl	$CHCl_3$	256	Deep purplish red	2.5RP 3/8
A.5	Codeine[a]	$CHCl_3$	225	Very dark purple	7.5P 2/4
A.5	D-Amphetamine HCl[a]	$CHCl_3$	35 to 44	Strong reddish orange Dark reddish brown	10R 6/12 to 7.5R 2/4
A.5	D-Methamphetamine HCl[a]	$CHCl_3$	36 to 44	Deep reddish orange Dark reddish brown	10R 4/12 to 7.5R 2/4
A.5	Diacetylmorphine HCl[a]	$CHCl_3$	256	Deep purplish red	7.5RP 3/10
A.5	Dimethoxy-meth HCl	$CHCl_3$	107	Moderate olive	7.5Y 5/8
A.5	Doxepin HCl	$CHCl_3$	21	Blackish red	7.5R 2/2
A.5	Dristan	Powder	20	Dark grayish red	5R 3/2
A.5	Exedrine	Powder	16	Dark red	5R 3/8
A.5	LSD	$CHCl_3$	114	Olive Black	10Y 2/2
A.5	Mace[b]	Crystals	87	Moderate yellow	7Y 7/8
A.5	MDA HCl[a]	$CHCl_3$	267	Black	Black
A.5	Meperidine HCl	$CHCl_3$	56	Deep brown	5YR 3/6
A.5	Mescaline HCl[a]	$CHCl_3$	50	Strong orange	5YR 6/12
A.5	Methadone HCl	$CHCl_3$	28	Light yellowish pink	2.5YR 8/4
A.5	Methylphenidate HCl	$CHCl_3$	71	Moderate orange yellow	10YR 8/8
A.5	Morphine monohydrate[a]	$CHCl_3$	239	Very deep reddish purple	10P 3/6
A.5	Opium[a]	Powder	47	Dark grayish reddish brown	10R 3/2
A.5	Oxycodone HCl[a]	$CHCl_3$	214	Pale violet	2.5P 6/4
A.5	Propoxyphene HCl	$CHCl_3$	230	Blackish Purple	2.5RP 2/2
A.5	Sugar	Crystals	59	Dark brown	5YR 2/4
A.6	Acetaminophen	$CHCl_3$	67	Brilliant orange yellow	2.5Y 8/12
A.6	Chlorpromazine HCl	$CHCl_3$	98	Brilliant greenish yellow	7.5Y 8.5/10
A.6	Codeine[a]	$CHCl_3$	101	Light greenish yellow	7.5Y 9/6
A.6	Diacetylmorphine HCl[a]	$CHCl_3$	89	Pale yellow	5Y 9/6
A.6	Dimethoxy-meth HCl	$CHCl_3$	82	Very yellow	2.5Y 8/14
A.6	Doxepin HCl	$CHCl_3$	83	Brilliant yellow	5Y 8.5/8
A.6	Dristan	Powder	51	Deep orange	5YR 6/12
A.6	Exedrine	Powder	67	Brilliant orange yellow	2.5Y 8/12
A.6	LSD	$CHCl_3$	55	Strong brown	5YR 5/10
A.6	Mace[b]	Crystals	102	Moderate greenish yellow	10Y 7/6
A.6	MDA HCl	$CHCl_3$	101	Light greenish yellow	7.5Y 9/6

Table 2. Continued

	Analyte	Solvent	ICSS-NBS[e]	Color	Munsell
A.6	Mescaline HCl[a]	$CHCl_3$	16	Dark red	5R 3/6
A.6	Morphine monohydrate[a]	$CHCl_3$	67	Brilliant orange yellow	2.5Y 8/12
A.6	Opium[a]	Powder	72	Dark orange yellow	10YR 6/10
A.6	Oxycodone HCl	$CHCl_3$	83	Brilliant yellow	5Y 8.5/8
A.7	LSD[a]	$CHCl_3$	219	Deep Purple	7.5P 3/10
A.8	Acetaminophen	MEOH	103	Dark greenish yellow	10Y 6/10
A.8	Baking soda	Powder	51	Deep orange	5YR 6/14
A.8	Chlorpromazine HCl	MEOH	48	Very orange	5YR 7/14
A.8	Dristan	Powder	200	Moderate purplish blue	10PB 4/2
A.8	Exedrine	Powder	200	Moderate purplish blue	10PB 4/2
A.8	Morphine monohydrate[a]	MEOH	146	Dark green	5G 3/6
A.9	Aspirin	Powder	228	Grayish purple	7.5P 5/2
A.9	Chlorpromazine HCl	$CHCl_3$	14	Very deep red	5R 3/10
A.9	Codeine[a]	$CHCl_3$	147	Very dark green	7.5G 2/6
A.9	Contac	Powder	95	Moderate olive brown	2.5Y 4/6
A.9	Diacetylmorphine HCl[a]	$CHCl_3$	256	Deep purplish red	5RP 3/10
A.9	Dimethoxy-meth HCl	$CHCl_3$	115	Very yellow green	5GY 6/10
A.9	Doxepin HCl	$CHCl_3$	41	Deep reddish brown	7.5R 2/8
A.9	Dristan	Powder	163	Light bluish green	5BG 7/6
A.9	Exedrine	Powder	177		10B 6/10
A.9	LSD	$CHCl_3$	120	Moderate yellow green	5GY 6/6
A.9	Mace[f]	Crystals	70	Light olive yellow	10YR 8/8
A.9	MDA HCl[a]	$CHCl_3$	157	Greenish black	7.5G 2/2
A.9	Morphine monohydrate[a]	$CHCl_3$	256	Deep purplish red	5RP 3/10
A.9	Opium[a]	Powder	65	Brownish black	7.5R 2/2
A.9	Oxycodone HCl	$CHCl_3$	84	Strong yellow	2.5Y 7/10
A.9	Propoxyphene HCl	$CHCl_3$	20	Dark grayish red	2.5R 3/2
A.9	Sugar	Crystals	83	Brilliant yellow	5Y 8.5/8
A.10	Chlorpromazine HCl	$CHCl_3$	21	Blackish red	5R 2/2
A.10	Codeine[a]	$CHCl_3$	166	Very dark bluish green	2.5BG 2/4
A.10	Contac	Powder	95	Moderate olive brown	2.5Y 4/6
A.10	Diacetylmorphine HCl[a]	$CHCl_3$	161	Deep bluish green	2.5BG 3/8
A.10	Dimethoxy-meth HCl	$CHCl_3$	59	Dark brown	5YR 2/4
A.10	Doxepin HCl	$CHCl_3$	17	Very dark red	5R 2/4
A.10	Dristan	Powder	94	Light olive brown	2.5Y 6/10
A.10	Exedrine	Powder	91	Dark grayish yellow	5Y 6/4
A.10	Hydrocodone tartrate	$CHCl_3$	165	Dark bluish green	5BG 3/6
A.10	LSD	$CHCl_3$	157	Greenish black	7.5G 2/2
A.10	Mace[f]	Crystals	111	Dark grayish olive	10Y 3/4
A.10	MDA HCl[a]	$CHCl_3$	166	Very dark bluish green	2.5BG 2/4
A.10	Mescaline HCl[a]	$CHCl_3$	107	Moderate olive	7.5Y 5/8
A.10	Morphine monohydrate[a]	$CHCl_3$	166	Very dark bluish green	2.5BG 2/4
A.10	Nutmeg	Extract	65	Brownish Black	10YR 2/2
A.10	Opium[a]	Powder	114	Olive black	10Y 2/2
A.10	Oxycodone HCl	$CHCl_3$	107	Moderate olive	7.5Y 5/8
A.10	Propoxyphene HCl	$CHCl_3$	41	Deep reddish brown	10R 2/6
A.10	Sugar	Crystals	98	Brilliant greenish yellow	10Y 8.5/10

Table 2. Continued

	Analyte	Solvent	ICSS-NBS[g]	Color	Munsell
A.11	Baking soda	Powder	181	Light blue	2.5PB 7/6
A.11	Exedrine	Powder	144	Light green	5G 7/6
A.11	Pentobarbital[a]	$CHCl_3$	222	Light purple	7.5P 7/6
A.11	Phenobarbital[a]	$CHCl_3$	222	Light purple	7.5P 7/6
A.11	Secobarbital[a]	$CHCl_3$	222	Light purple	7.5P 7/6
A.11	Tea	Extract	120	Moderate yellow green	2.5GY 7/8
A.11	Tobacco	Extract	136	Moderate yellowish green	10GY 6/6
A.12	D-Methamphetamine HCl[a]	$CHCl_3$	183	Dark blue	2.5PB 2/6
A.12	Dimethoxy-meth HCl[a]	$CHCl_3$	179	Deep blue	2.5PB 3/8
A.12	MDMA HCl	$CHCl_3$	183	Dark blue	2.5PB 2/6
A.12	Methylphenidate HCl	$CHCl_3$	214	Pale Violet	2.5P 6/4

[a] Usual kit reagent for that particular drug.
[b] Aqueous phase.
[c] Aqueous phase after chloroform extraction.
[d] Chloroform phase (marijuana extraction usually rapid compared to other materials).
[e] Not extracted into chloroform.
[f] 2-Chloroacetophenone.
[g] Abbreviations: ICSS-NBS=Inter-Society Color Council and the National Bureau of Standards, Munsell=Munsell color notation, $CHCl_3$=chloroform, EtOH=ethanol, MEOH=methanol, B=blue, G=green, P=purple, Y=yellow, R=red.

(A.9) and Mecke (A.10) reagents. Unlike the cobalt thiocyanate reaction, different colors were produced with different drugs making it easier to presumptively identify the specific drug present. For example, a selected battery of tests to identify heroin (diacetylmorphine) might include the Mandelin, Marquis and Froehde tests because they would produce reddish brown, deep purplish red and purplish red colors, respectively. Codeine, a second opiate, could be identified with the same battery of CSTs because it produced olive, dark purple and dark green colors, respectively. These three CSTs were reactive to many opioids with LODs as low as 1–5 μg, Table 4, but as mentioned the colors produced and the sensitivity was dependent on the many factors listed above.

Since positive reactions were dependent on the functional groups present in the chemical structure of the tested analytes, several of the CSTs were specific for certain classes of drugs. The *p*-DMAB reagent (A.7) reacted only with LSD, producing a deep purple color. This test had an LOD of 6 μg for LSD. Although mace, nutmeg and tea reacted with the modified Duquénois–Levine test (A.3), as shown in Table 2, only tetrahydrocannabinal (THC) produced a deep purple color that was extracted into chloroform. The Simon's test is reported to be specific for secondary amines like methamphetamine and MDMA. It did not react with ephedrine or pseudoephedrine because their structure contains an hydroxyl group that is in close proximity to the amine. Barbiturates can be detected by both the Dille–Koppanyi (A.2) and Zwikker (A.11) reagents. However, the Dille–Koppanyi test was more sensitive with LODs of 25 μg or lower whereas the LOD for phenobarbital with the Zwikker test was 1000 μg.

Table 3
Specificity of color tests. (+) Indicates that a color reaction occurs[a]

	Reagent											
	A.1	A.2	A.3	A.4	A.5	A.6	A.7	A.8	A.9	A.10	A.11	A.12
Acetaminophen	–	–	–	+	–	+	–	+	–	–	–	–
Alprazolam	–	–	–	–	–	–	–	–	–	–	–	–
Aspirin	–	–	–	+	+	–	–	–	+	–	–	–
Baking soda	–	–	–	–	–	–	–	+	–	–	+	–
Brompheniramine maleate	+	–	–	+	–	–	–	–	–	–	–	–
Chlordiazepoxide HCl	+	–	–	–	–	–	–	–	–	–	–	–
Chlorpromazine HCl	+	–	–	+	+	+	–	+	+	+	–	–
Contac	–	–	–	+	–	–	–	–	+	+	–	–
Diazepam	–	–	–	–	–	–	–	–	–	–	–	–
Doxepin HCl	+	–	–	+	+	+	–	–	+	+	–	–
Dristan	–	–	–	+	+	+	–	+	+	+	–	–
Ephedrine HCl	+	–	–	–	–	–	–	–	–	–	–	–
Exedrine	–	–	–	+	+	+	–	+	+	+	+	–
Hydrocodone tartrate	+	–	–	–	–	–	–	–	–	+	–	–
Mace[b]	–	–	+	+	+	+	–	–	+	+	–	–
Meperidine HCl	+	–	–	–	+	–	–	–	–	–	–	–
Methaqualone	–	–	+	–	–	–	–	–	–	–	–	
Methylphenidate HCl	+	–	–	+	+	–	–	–	–	–	–	+
Nutmeg[b]	–	–	+	–	–	–	–	–	–	+	–	–
Phencyclidine HCl	+	–	–	–	–	–	–	–	–	–	–	–
Propoxyphene HCl	+	–	–	+	+	–	–	–	+	+	–	–
Pseudoephedrine HCl	+	–	–	–	–	–	–	–	–	–	–	–
Quinine HCl	+	–	–	+	–	–	–	–	–	–	–	–
Salt	–	–	–	+	–	–	–	–	–	–	–	–
Sugar	–	–	–	–	+	–	–	–	+	+	–	–
Tea[b]	–	–	+	–	–	–	–	–	–	–	+	–
Tobacco	–	–	–	–	–	–	–	–	–	–	+	–

[a] Substances that gave no colors with these reagents are: D-galactose, glucose, mannitol, oregano, rosemary and thyme.

[b] Tea, mace and nutmeg may interfere with the Duquenios test, but not the Duquenois–Levine modified test (A.3).

4. Conclusions

Chemical spots tests are valuable tools for the presumptive identification of drugs in unknown samples. These tests are very sensitive with LODs typically 1 to 50 μg depending on the CST and the analyte. The methods and validation procedures for 12 chemical spot tests for use in the laboratory or in the field were described. For the identification of unknown drugs, reference colors from the Munsell and ICSS-NBS centroid color charts representing positive reactions for the 12 CSTs were included. Although these tests are sensitive and can be relatively specific, the actual color observed by the analyst performing the CST depends on many factors such as the concentration of the drug, whether the drug is a salt or free base, which salt form is

Table 4
Drug detection limits[a]

Reagent	Analyte	Drug detection limit (μg)
A.1	Cocaine HCl	60
A.1	Methadone HCl	250
A.2	Amobarbital	25
A.2	Pentobarbital	10
A.2	Phenobarbital	15
A.2	Secobarbital	25
A.3	THC	5
A.4	D-Amphetamine HCl	20
A.4	D-Methamphetamine HCl	100
A.4	Codeine	20
A.4	Diacetylmorphine HCl	20
A.4	Morphine monohydrate	5
A.5	D-Amphetamine HCl	10
A.5	Codeine	1
A.5	Diacetylmorphine HCl	10
A.5	LSD	5
A.5	Mescaline HCl	10
A.5	Methadone HCl	20
A.5	D-Methamphet HCl	5
A.5	Morphine monohydrate	5
A.6	Mescaline HCl	1
A.7	LSD	6
A.8	Morphine monohydrate	200
A.9	Codeine	50
A.9	Diacetylmorphine HCl	200
A.9	LSD	50
A.9	Mescaline HCl	100
A.9	Morphine monohydrate	25
A.10	Codeine	25
A.10	Diacetylmorphine HCl	200
A.10	LSD	50
A.10	Mescaline HCl	50
A.10	Morphine monohydrate	50
A.11	Phenobarbital	1000
A.12	D-Methamphetamine HCl	10
A.12	Methylphenidate HCl	300

[a] The solvent was chloroform except for A.8 which was methanol.

present, the presence of contaminants in the sample, the color discrimination of the analyst and the conditions under which the CST is performed.

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